Adipose tissue isomeric trans fatty acids and risk of myocardial infarction in nine countries: the EURAMIC study

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Abstract: Dietary isomeric trans fatty acids-mainly produced by hydrogenation of oils-are suspected of increasing the risk of coronary heart disease. Dietary trans fatty acid intake is reflected in the fatty acid composition of adipose tissue. In an international multicentre study in eight European countries and Israel (EURAMIC), adipose tissue aspiration samples were obtained from 671 men with acute myocardial infarction (AMI), aged 70 years or less, and 717 men without a history of AMI (controls). The proportion of fatty acids, including isomeric trans monoenoic fatty acids with 18 carbon atoms (C18:1), was determined by gas chromatography. Although there were considerable differences between countries in mean (SD) proportion of adipose tissue C18:1 trans fatty acids, there was no overall difference between cases (1.61 [0.92]%) and the controls (1.57 [0.86]%). The risk of AMI did not differ significantly from 1.0 over quartiles of adipose C18:1 trans fatty acids: the multivariate odds ratio was 0.97 (95% CI 0.56-1.67) for the highest versus lowest quartile. After exclusion of subjects from Spanish centres because they had far lower proportions of adipose trans fatty acids than subjects from other countries, there was a tendency to increased risk of AMI in the upper quartiles of C18:1 trans; however, the trend was not statistically significant. Our results reflect considerable differences between countries in dietary intake of trans fatty acids but do not suggest a major overall effect of C18:1 trans fatty acids on risk of AMI. We cannot exclude the possibility that trans fatty acids have a significant impact on risk of AMI in populations with high intake.

DOI: https://doi.org/10.1016/S0140-6736(95)90273-2

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Summary

Dietary isomeric *trans* fatty acids—mainly produced by hydrogenation of oils—are suspected of increasing the risk of coronary heart disease. Dietary *trans* fatty acid intake is reflected in the fatty acid composition of adipose tissue. In an international multicentre study in eight European countries and Israel (EURAMIC), adipose tissue aspiration samples were obtained from 671 men with acute myocardial infarction (AMI), aged 70 years or less, and 717 men without a history of AMI (controls). The proportion of fatty acids, including isomeric *trans* monoenoic fatty acids with 18 carbon atoms (C18:1), was determined by gas chromatography.

Although there were considerable differences between countries in mean (SD) proportion of adipose tissue C18:1 *trans* fatty acids, there was no overall difference between cases (1·61 [0·92]%) and the controls (1·57 [0·86]%). The risk of AMI did not differ significantly from 1·0 over quartiles of adipose C18:1 *trans* fatty acids: the multivariate odds ratio was 0·97 (95% CI 0·56–1·67) for the highest versus lowest quartile. After exclusion of subjects from Spanish centres because they had far lower proportions of adipose *trans* fatty acids than subjects from other countries, there was a tendency to increased risk of AMI in the upper quartiles of C18:1 *trans*; however, the trend was not statistically significant.

Our results reflect considerable differences between countries in dietary intake of *trans* fatty acids but do not suggest a major overall effect of C18:1 *trans* fatty acids on risk of AMI. We cannot exclude the possibility that *trans* fatty acids have a significant impact on risk of AMI in populations with high intake.

Lancet 1995; **345:** 273–78

See Commentary page 269

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Introduction

Isomeric unsaturated fatty acids containing one or more double bonds in the *trans* position are formed concomitantly with saturated fatty acids when vegetable oils or fish oils are hardened by hydrogenation. Most dietary *trans* fatty acids are found in margarines, dressings, and other fat products that contain hydrogenated fats. Isomeric fatty acids are also formed in the intestinal tract of ruminants and they appear in small amounts in milk fat and beef fat. Several studies have shown that dietary *trans* fatty acids increase serum total cholesterol or low-density-lipoprotein (LDL) cholesterol concentration by comparison with nonhydrogenated vegetable oils.[1–7] In some studies, a reduction in high-density-lipoprotein (HDL) cholesterol concentration by *trans* fatty acids has been found,[3,4] whereas in others no effect on HDL-cholesterol has been observed.[2,5–7] *Trans* fatty acids may also increase the concentration of lipoprotein (a), which is considered to be a risk factor for coronary heart disease.[5,8]

The observed effects of dietary trans fatty acids on serum lipoproteins may increase the risk of coronary heart disease,[9] but amounts of trans fatty acids that have been used in clinical trials have usually been greater than those consumed in everyday life. In a prospective study of a large group of women in the USA, the intake of *trans* fatty acids as estimated from dietary questionnaires was related to increased risk of coronary heart disease during 8 years of follow-up,[10] and in a case-control study in the USA dietary intake of *trans* fatty acids was similarly related to increased risk of myocardial infarction.[11] To investigate the role of *trans* fatty acids in coronary heart disease we analysed adipose tissue *trans* fatty acids—known to be derived from the diet[12]—in a multicentre case-control study of men with acute myocardial infarction (AMI) from eight European countries and Israel, representing populations with varying dietary habits.

Subjects and methods

Subjects

Subjects were men aged 70 years or younger from ten study centres in nine countries, examined during 1991 and 1992 (table 1). Cases were 742 men with first AMI—confirmed by characteristic electrocardiographic and serum enzyme changes—who were admitted to hospital within 24 h of manifesting symptoms. Cases were consecutively recruited from the coronary care units of participating hospitals. Control subjects were 757 men without a history of AMI, recruited from the population in the catchment area and frequency-matched for age according to 5-year intervals. Whenever possible, random samples from local population registries were used (Finland, Israel, Germany, UK, Switzerland). In some centres (Russia, Spain), population registries could not be used because of incomplete coverage or legal restrictions, so hospital controls were selected who had

Centre	Cases				Controls				
	No studied	Response (%)*	Mean age	No with *trans* fatty acid analysis	No studied	Response (%)*	Mean age	No with *trans* fatty acid analysis	Method of recruitment
Helsinki, Finland	62	97	52·8	57	61	51	53·7	59	PR
Berlin, Germany	77	82	56·9	56	97	73	52·8	95	PR
Jerusalem, Israel	59	60	54·8	50	60	53	54·8	58	PR
Zeist, Netherlands	72	75	53·5	67	63	50	52·0	58	GP, PR
Sarpsborg, Norway	101	96	55·3	98	102	98	55·5	100	FR
Moscow, Russia	100	97	54·0	87	100	79	47·8	94	GP, H
Edinburgh, UK	58	98	54·6	58	43	61	55·0	41	PR
Granada, Spain	57	45	54·5	54	54	67	55·0	52	H
Malaga, Spain	100	89	54·6	91	102	77	55·2	96	GP, H
Zurich, Switzerland	57	93	56·3	53	74	26	53·5	64	PR

*Based on eligible subjects. PR=population register, GP=general practitioners, H=hospital controls, FR=friends and relatives.

Table 1: **Subjects, response rates, and methods of control recruitment**

diseases not known to be associated with dietary factors (renal colic, non-infectious prostatism, acute appendicitis, non-infectious ear disease, hernia, volvulus, rectal or anal disease except cancer, haemorrhoids, or chronic infection). Where it was thought that low response rates from population-based samples would spoil internal validity, control subjects were selected from the catchment area via a random sample by the patients' general practitioners (Netherlands) or by inviting friends and relatives of the case (Norway). In the Netherlands, Spain, and Russia, methods of subjects recruitment were combined (table 1).

Subjects in both groups were residents of their countries, they had not changed their diet for health reasons, and they had not lost more than 5 kg in weight in the past year. Subjects with a history of alcohol or drug abuse, major psychiatric disorder, and those who were institutionalised were excluded from the study. Informed consent was obtained in accordance with the ethical standards of the responsible committees on human experimentation. The study design has been reported in detail elsewhere.[13]

Samples

A needle aspiration biopsy specimen was obtained from the subcutaneous adipose tissue of the buttock, as described by Beynen and Katan.[14] In cases, sample was taken within 7 days of hospital admission. The samples were immediately frozen on dry ice and stored at −70°C in the original plastic adaptors. Needle biopsies were done on 1499 subjects, 742 cases and 757 controls. In 31 samples (27 cases, 4 controls) no fat aspirate was obtained, and in a further 52 samples (24 cases, 28 controls) the fatty acid composition could not be determined. In 28 (20 cases, 8 controls) of the remaining 1416 samples, too little material was available (less than 5 mg total fatty acids) to analyse the *trans* fatty acid content. Therefore, results for *trans* fatty acids were available for 671 cases and 717 control subjects. Samples had a mean (SD) weight of 29 (16) mg and contained 19 (11) mg fatty acids. A nonfasting blood sample was drawn; in cases this was done within 24 h of hospital admission. Information on smoking was collected by standard questionnaires, and that on socioeconomic status, family history of heart disease, and alcohol intake by locally developed questionnaires.

Methods

The adipose tissue and serum samples were transported to the coordinating centre in Zeist on dry ice, which guaranteed a temperature of −56°C. Pooled samples were included in shipments and processed in a similar manner as the actual samples to control for transport and storage conditions. In Zeist, the adipose tissue samples were saponified and divided into two parts, one for the analysis of antioxidant vitamins[15] and the other for the analysis of fatty acid composition.

Assays for fatty acids and serum lipids were done in Finland at the National Public Health Institute. In the fatty acid analysis the saponified sample was acidified with HCl and free fatty acids were extracted with hexane and methylated with acidic methanol. Fatty acid patterns were determined by gas chromatography (HNU Nordion Oy, Finland, HRCG 412). Fatty acid peaks from C12:0 to C22:6 were identified by computer in a temperature-programmed run. From the chromatograms, the C18:1 *trans* fatty acids were identified as one sum peak because with the temperature program used the column did not give sharp separation of different positional *trans*-isomers but only a block of peaks. *Trans* fatty acids with 16 and 20–22 carbon atoms and the different C18:2 *cis/trans* isomers were below the detection limit in most samples.

Among cases and control subjects, the percentages with major risk factors and possible confounders were calculated for the different centres, as were means (and SDs) of the proportion of C18:1 *trans* fatty acids. Differences within centres and 95% CIs were calculated; the center-adjusted overall mean difference (and standard deviation) was estimated by linear regression. For significance testing, fatty acid values were log-transformed.

Potential confounders or effect modifiers were identified with stratified analysis. We calculated the centre-adjusted partial correlation coefficients for the association of *trans* fatty acids with potential confounders. For multivariate analysis, multiple logistic regression was used with maximum likelihood estimation of the regression coefficients. Relative risks were estimated as odds ratios (ORs) in quartiles compared with the lowest quartile, based on the distribution among control subjects. The fitted model included age, centre, smoking, and body mass index (BMI); socioeconomic status was not included because it showed no additional effect on ORs. Smoking categories included never smokers, ex-smokers, pipe/cigar smokers, and current cigarette smokers, the last category being further divided into subjects smoking less than 5, 6–10, 11–20, and over 20 cigarettes per day. Interactions between C18:1 *trans* fatty acids and centre were examined by including a product term in the logistic model. Moreover, ORs for the different quartiles compared with the lowest quartile were calculated separately for each centre, based on the centre-specific distribution in control subjects. To evaluate potential bias caused by the different methods of selecting control subjects, the multivariate model was re-analysed to include only centres with population control subjects. This analysis had only a marginal effect on the estimated risk in the different quartiles. The data were also re-analysed after exclusion of the two Spanish centres.

Centre	No of cases/ controls	Mean (SD) C18 *trans* fatty acids (%)		
		Cases	Controls	Difference (95% CI)
Helsinki, Finland	57/59	1·77 (0·74)	1·51 (0·63)	0·26 (0·01 to 0·51)
Berlin, Germany	56/95	1·44 (0·51)	1·38 (0·45)	0·06 (−0·10 to 0·22)
Jerusalem, Israel	50/58	2·06 (0·63)	2·15 (0·71)	−0·09 (−0·34 to 0·16)
Zeist, Netherlands	67/58	2·25 (0·73)	2·43 (0·87)	−0·18 (−0·46 to 0·10)
Sarpsborg, Norway	98/100	2·41 (0·83)	2·15 (0·69)	0·26 (0·05 to 0·47)
Moscow, Russia	87/94	1·50 (0·47)	1·68 (0·56)	−0·18 (−0·33 to −0·03)
Edinburgh, UK	58/41	2·25 (0·55)	2·22 (0·62)	0·03 (−0·20 to 0·26)
Granada, Spain	54/52	0·42 (0·36)	0·43 (0·27)	−0·01 (−0·13 to 0·11)
Malaga, Spain	91/96	0·40 (0·23)	0·47 (0·29)	−0·07 (−0·14 to 0·00)
Zurich, Switzerland	53/64	1·69 (0·60)	1·72 (0·43)	−0·03 (−0·22 to 0·16)
All centres*	671/717	1·60	1·59	0·01 (−0·02 to 0·04)

*Centre-adjusted.

Table 2: **Proportions of adipose tissue C18 *trans* fatty acids in men with AMI and controls**

Centre	% smokers			Mean BMI			% low socioeconomic status		
	Cases	Controls	95% CI for difference	Cases	Controls	95% CI for difference	Cases	Controls	95% CI for difference
Helsinki, Finland	63	25	23 to 53	27·1	26·6	−1·0 to 2·0	47	29	1 to 35
Berlin, Germany	52	38	−2 to 30	26·5	25·7	−0·3 to 1·9	11	3	−1 to 17
Jerusalem, Israel	52	33	1 to 37	25·7	26·2	0·6 to 2·2	8	12	−15 to 7
Zeist, Netherlands	55	38	0 to 34	26·9	25·4	−0·6 to 1·6	49	45	−14 to 22
Sarpsborg, Norway	60	23	24 to 50	25·9	24·9	0·0 to 2·0	56	32	11 to 37
Moscow, Russia	59	39	6 to 34	26·2	25·7	−0·4 to 1·4	4	5	−7 to 5
Edinburgh, UK	48	27	2 to 40	26·8	26·3	−0·9 to 1·9	21	12	−5 to 23
Granada, Spain	76	54	−4 to 40	26·4	26·2	−1·2 to 1·6	2	15	−23 to −3
Malaga, Spain	74	32	29 to 55	27·9	27·3	−0·6 to 1·8	30	43	−27 to 1
Zurich, Switzerland	37	14	7 to 39	26·4	25·6	−1·4 to 1·0	10	8	−9 to 13
All centres*	59	33	21 to 30	26·5	26·0	0·2 to 0·9	22	18	0 to 8

*Centre-adjusted.

Table 3: **Smoking, BMI, and socioeconomic status of men with AMI and controls**

Results

Subjects from the different centres varied considerably in mean proportions of adipose tissue C18:1 *trans* fatty acids (table 2), ranging in cases from 0·40% in Malaga to 2·41% in Sarpsborg, and in control subjects from 0·43% in Granada to 2·43% in Zeist. There was no overall difference between cases and controls. In Norway and Finland, cases had significantly higher mean proportions of C18:1 *trans* fatty acids than controls.

Cases were older (mean [SD] 54·6 [9·1] years) than control subjects (53·2 [9·2] years; $p<0·05$). There were more smokers among cases than among controls (table 3), but consumers of alcohol were equally distributed between cases and controls (80% and 82%). Cases had slightly higher BMI than controls, and low socioeconomic status was more common in cases. Alcohol intake showed a significant positive centre-adjusted partial correlation with C18:1 *trans* fatty acids ($r=0·15$, $p<0·001$), whereas BMI ($r=-0·12$, $p<0·01$) and socioeconomic status ($r=-0·07$, $p<0·025$) showed inverse correlations. Age ($r=0·07$) and smoking ($r=0·04$) did not correlate significantly with C18:1 *trans* fatty acids.

The OR of AMI was not significantly different from 1·0 in quartiles of *trans* fatty acids classified according to the distribution in control subjects (table 4). Recalculations of the ORs after exclusion of centres with non-population-based control subjects and of subjects with previous angina pectoris did not affect significantly the results (not shown). Nearly all cases and controls from the two Spanish centres had proportions of C18:1 *trans* fatty acids in the lowest quartile, in which almost no overlap occurred with the distribution in other countries. Therefore, the ORs by quartiles were recalculated after exclusion of the Spanish groups (table 5). After exclusion, the OR for AMI in the second highest quartile of *trans* fatty acids was significantly higher than in the lowest quartile, but the trend over quartiles was not significant (χ^2 for trend 0·38, $p=0·54$). Adjustment for confounding factors did not further modify the result.

We evaluated whether the association between *trans* fatty acids and AMI was homogeneous among centres. The interaction term *trans* fatty acids (expressed as a continuous variable) multiplied by centre was significant (χ^2 19·2, $p<0·05$). Table 6 shows the ORs for AMI, adjusted for age, BMI, smoking and socioeconomic status, by quartiles and calculated separately for centres. ORs were highest and significantly increased compared with the lowest quartile in Norway and Finland, and lowest and significantly decreased in Russia and Spain. The range between highest and lowest quartiles differed among centres, but the observed ORs were clearly not associated with this range.

Oleic acid ($r=-0·68$), linoleic acid ($r=0·19$), and arachidonic acid ($r=-0·24$) showed statistically significant correlations with C18:1 *trans*. Inclusion of these fatty acids in the model that contained age, centre, smoking, and BMI did not change the ORs, which indicates that there was no confounding effect by fatty acids on the association between C18:1 *trans* fatty acids and AMI. After exclusion of the Spanish subjects, the correlation between oleic acid and C18:1 *trans* was somewhat reduced but remained statistically significant ($r=-0·44$, $p<0·001$).

	Quartiles*			
	Q1	Q2	Q3	Q4
Median of C18:1 *trans* (%)	0·45	1·29	1·80	2·51
No cases/controls	182/181	125/178	182/179	182/179
Crude OR	1·00	0·70	1·01	1·01
Center-adjusted OR	1·00	0·65	0·85	0·78
Multivariate OR† (95% CI)	1·00	0·68 (0·41–1·13)	1·05 (0·63–1·75)	0·97 (0·56–1·67)

*Cutpoints: 1·00, 1·55, 2·15%; †adjusted for age, centre, smoking and BMI, and slightly different numbers of cases and control subjects were used due to missing values for the confounders.

Table 4: **Risk of acute myocardial infarction in quartiles of C18:1 *trans* fatty acid distribution of control subjects**

	Quartiles*			
	Q1	Q2	Q3	Q4
Median of C18:1 *trans* (%)	1·12	1·55	1·98	2·63
No cases/controls	108/143	113/143	158/140	147/143
Crude OR (95% CI)	1·00	1·05 (0·74–1·49)	1·49 (1·07–2·10)	1·36 (0·97–1·91)
Center-adjusted OR (95% CI)	1·00	1·00 (0·70–1·43)	1·32 (0·91–1·91)	1·14 (0·77–1·67)
Multivariate OR† (95% CI)	1·00	1·16 (0·79–1·71)	1·53 (1·02–2·28)	1·44 (0·94–2·20)

*Cutpoints: 1·34, 1·74, 2·27%; †adjusted for age, centre, smoking and BMI.

Table 5: **Risk of acute myocardial infarction in quartiles of C18:1 *trans* fatty acid distribution of control subjects after exclusion of two Spanish centres**

Discussion

The fatty acid composition of adipose tissue is an appropriate biomarker of dietary intake for those fatty acids that are not synthesised by human beings—ie, for linoleic acid, n-3 polyunsaturated fatty acids, and for isomeric *trans* fatty acids.[12,16–18] In the study by London et al[17] in women, the Spearman correlation between *trans* fatty acids intake and their proportion in the adipose tissue was 0·51, which was in the same order as the correlations between the intakes of n-6 and n-3 polyunsaturated fatty acids and their proportions in adipose tissue. In another US study in men,[18] the

Centre	Quartiles				95% CI (Q4 vs Q1)	χ^2 for trend (p)†	p (87·5)–p (12·5)‡
	Q1	Q2	Q3	Q4			
Sarpsborg, Norway	1·0	2·7	2·3	5·4	1·5 to 13·1	6·57 (0·01)	1·24
Helsinki, Finland	1·0	2·5	2·0	5·0	1·3 to 19·6	4·72 (0·02)	1·49
Berlin, Germany	1·0	0·9	0·9	1·8	0·7 to 5·2	1·40 (0·24)	0·71
Edinburgh, UK	1·0	0·6	0·8	1·6	0·4 to 6·6	0·58 (0·45)	1·29
Zurich, Switzerland	1·0	0·3	0·8	1·5	0·5 to 4·9	1·06 (0·30)	0·98
Jerusalem, Israel	1·0	2·1	1·5	0·8	0·2 to 3·2	0·32 (0·57)	1·78
Zeist, Netherlands	1·0	1·8	0·5	0·8	0·2 to 2·6	1·02 (0·31)	2·02
Malaga, Spain	1·0	1·2	1·3	0·3	0·1 to 1·0	2·63 (0·11)	0·52
Moscow, Russia	1·0	1·1	0·5	0·2	0·1 to 0·7	7·00 (0·01)	1·36
Granada, Spain	1·0	0··5	0·5	0·2	0·0 to 0·6	6·51 (0·01)	0·51

*OR adjusted for age, BMI, smoking, and socioeconoomic status; †quartiles included in model as continuous variables; ‡median of Q4–median of Q1.

Table 6: **Multivariate risk* of acute myocardial infarction in quartiles of C 18:1 *trans* fatty acid distribution of control subjects in individual centres**

corresponding correlation for *trans* fatty acids was 0·29–0·34, compared with 0·47 and 0·48 for linoleic and eicosapentaenoic acids, respectively.

The view that intake of *trans* fatty acids is associated with the risk of coronary heart disease is not supported by the lack of significant difference in adipose tissue *trans* fatty acids between patients with AMI and control subjects in the whole study population. Thomas et al from the UK[19] compared postmortem adipose tissue samples from patients who died of coronary heart disease with controls who had died from other causes, and found higher adipose tissue C16:1 *trans* fatty acid levels and a higher C16:1 *trans* to linoleic acid ratio in coronary heart disease patients than controls. C18:1 *trans* fatty acids were also higher in patients than controls but not statistically significantly so. Willett et al[10] reported a higher estimated *trans* fatty acid intake in the year 1980 in women who subsequently had new coronary heart disease events during an 8-year follow-up. No biological markers of fatty acid intake were determined, but dietary intake data suggested that the increased risk was associated with use of fats containing hydrogenated vegetable oils, and a case-control study reported by the same group[11] supported the relation between *trans* fatty acid intake, adjusted for age, sex, and energy intake, and risk of AMI. The importance of dietary *trans* fatty acids may well differ between countries due to differences in the origin and composition of fatty acid isomers and in other factors influencing the risk of coronary heart disease. In the study from the UK[19] hydrogenated marine oils might have affected the findings more than hydrogenated vegetable oils.

The source of controls and their response rates in our study might have affected their representativeness for the population from which the cases originated. Therefore, between-country/centre differences in OR may partly reflect variability in within-centre validity. However, because restriction of the overall analysis to centres with population controls, who had poorest response rates, did not alter the estimates, the source of controls is unlikely to have systematically affected the results.

Socioeconomic status was assessed in each centre with a questionnaire that was considered the most appropriate one for the local study population; this allowed adjustment for socioeconomic status within centre (eg, table 6) and explains the apparent differences in socioeconomic status score between subjects from different centres. Since adjustment for socioeconomic status did not appreciably alter the results it is unlikely that any major socioeconomic-status-related bias is still present in the data.

Our study involved two Spanish centres with 164 cases (24% of all cases) and 148 control subjects (21%) with an adipose tissue fatty acid pattern quite different from that found in other countries. The proportion of C18:1 *trans* fatty acids was very low and that of oleic acid high. The lowest quartile of the distribution of C18:1 *trans* consisted almost exclusively of cases and control subjects from Granada and Malaga. When Spanish subjects were excluded the relative risk of AMI was slightly greater in the upper quartiles of C18:1 *trans* than in the lowest quartile. Thus, although the overall association of C18:1 *trans* fatty acids with risk of AMI was not significant, the possibility remains that *trans* fatty acids contribute to the risk of AMI in countries with high intakes.

The significant inverse correlation between C18:1 *trans* fatty acids and oleic acid in the adipose tissue was partly due to the dietary pattern in Spain—ie, low intake of *trans* fatty acids combined with high oleic acid intake. Since the inverse association was found even after exclusion of Spanish subjects, a possible interpretation of this finding is that in adipose tissue C18:1 *trans* fatty acids tend to replace the corresponding fatty acid with *cis* configuration. By contrast with oleic acid, linoleic acid showed a significant positive correlation with C18:1 *trans*, which suggests that these fatty acids were to some extent derived from the same dietary sources. However, adjustment for oleic acid and linoleic acid did not affect the ORs for AMI in the *trans* fatty acid quartiles.

The fatty acid assay used in our study was designed primarily to include the most important dietary components and to give good separation between polyunsaturated fatty acids. It was not possible to separate different positional isomers of C18:1 fatty acids and to determine accurately C16, C18:2, and C20–22 *trans* isomers in most of the samples. Therefore, only total C18:1 *trans* fatty acids were included in the results. The findings of Ohlrogge et al[20] suggested that C18:1 *trans* fatty acids in the adipose tissue were mainly derived from hydrogenated vegetable oils. In agreement with this suggestion, a study of dietary fats in Finland[21] showed that C18:1 *trans* isomers were found in margarines made of vegetable oils; margarines that contained dairy fat had in addition C18:2 isomers, and C16:1, C20:1, and C22:1 *trans* isomers were found in products that incorporated hydrogenated fish oils. We can conclude from these findings that the C18:1 *trans* fraction of fatty acids in our study represented primarily intake of *trans* fatty acids from hydrogenated vegetable oils and to some extent from dairy fat. It is quite possible that different isomeric fatty acids have different effects with respect to risk of coronary heart disease. On the other hand, the C16 and C20–22 *trans* fatty acids were found in only very small

proportions. It is known that hydrogenated fish oils are consumed in the Netherlands, Norway, and UK,[22] but it is impossible to determine whether hydrogenated fish oils might have contributed to the higher than average proportions of C18:1 *trans* fatty acids found in these countries.

In Norway and Finland a significant positive association was found between *trans* fatty acids in adipose tissue and risk of AMI, whereas a tendency in the opposite direction was evident in Spain and in Russia. Overall there seemed to be no association between *trans* fatty acids and risk of AMI. Exclusion of the two Spanish centres with very low proportions of *trans* fatty acids altered the ORs for AMI in the remainder of the countries analysed together. Thus the possibility cannot be excluded that *trans* fatty acids influence the risk of AMI in countries with a western European lifestyle, in particular in Nordic contries, but not in the Mediterranean countries represented by Spain with its high oleic acid intake and Israel with its high intake of linoleic acid. However, there were considerable differences even between the western European countries as shown, for example, by the discordant findings in Norway and the Netherlands, the two countries with highest mean proportions of *trans* fatty acids in the adipose tissue. Differences in findings between countries can be interpreted as reflecting an interaction of *trans* fatty acids with some other dietary component(s), or as showing that *trans* fatty acid intake is not aetiologically related to AMI but serves rather as a marker of other dietary factors. For example, the protective effect in the Russian sample could reflect the absence of a deleterious dietary factor.

Because in man adipose tissue *trans* fatty acids are derived from the diet,[12] the differences between countries in mean proportions of adipose tissue *trans* fatty acids probably represent differences in mean intakes. In the studies from USA, adipose tissue total *trans* fatty acids made up 4·2–4·4% of total fatty acids and C18:1 *trans* fatty acids 2·9%. The corresponding mean intakes as assessed by a food-frequency method were estimated to be 4 g per day.[17,18] This is considerably less than the estimated mean intakes of 13·3 g per day[23] and 8·1 g per day[24] derived from US food supply data. European data on the intake of *trans* fatty acids is fragmentary, derived at different times, and based on varying methodology. Highest levels have been reported from the Netherlands (10 g per day[25]) and Norway (7–10 g per day[26]). Intakes in the UK (7 g per day[27]), Israel (6 g per day[28]), and Germany (5 g per day[29]) are similar. In Finland the estimated intake was 5·6 g per day in 1980 and 3 g per day in 1984. A study from Spain reported a mean daily intake of *trans* fatty acids of 2·4 g per day.[30] The proportion of C18:1 *trans* fatty acids in adipose tissue was in all the countries we studied lower than reported values from the USA. The values were also slightly lower than those reported in most previous studies.[22] This may reflect methodological differences or a general decline in the intake of isomeric *trans* fatty acids in European countries.

Our results do not indicate a major effect of adipose tissue C18:1 *trans* fatty acids on the risk of AMI in men. However, we cannot exclude the possibility that the contribution of *trans* fatty acids to risk of AMI is significant in countries with high intakes of *trans* fatty acids. Further evaluation of the situation within different countries is called for before strong recommendations are made.

Members of the EURAMIC project management group are J K Huttumen, L Kohlmeier, J Martin-Moreno, and F J Kok (project leader). The EURAMIC study was supported by an EC-Concerted Action by the Commission of European Communities. The national studies were financed by British Heart Foundation, Dutch Ministry of Health, Spanish FIS, German Federal Health Office, Norwegian Research Council, Russian Ministry of Science, Swiss NRF (grant 32-31312-91), and the Yrjö Jahnsson Foundation, Finland. We thank our numerous co-workers in the different countries for their contributions. Their names and addresses have been listed elsewhere.[15]

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Trans isomers of oleic and linoleic acids in adipose tissue and sudden cardiac death

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Summary

Trans isomers of unsaturated fatty acids are formed by biological or industrial hydrogenation. A population case-control study of sudden cardiac death in men was done to test the hypothesis that *trans* isomers of oleic acid and linoleic acid increase the risk of sudden cardiac death due to coronary artery disease.

In adipose tissue obtained at necropsy from 66 cases of sudden cardiac death and taken from 286 healthy age and sex matched controls, the proportions of *trans* isomers of oleic and linoleic acid were measured by gas-liquid chromatography. In cases, the mean (SE) percentage of total *trans* fatty acids (C18:1 plus C18:2), expressed as a proportion of all fatty acids, was significantly lower (2·68 [0·08]%) than in healthy controls (2·86 [0·04]%; $p<0·05$). *Trans* C18:1 was 2·1 (0·7)% in cases compared with 2·27 (0·04)% ($p<0·05$) in controls. The proportion of all *trans* isomers of linoleic acid was 0·58 (0·02)% in cases compared with 0·59 (0·01)% in controls ($p=0·98$). The estimated relative risk for sudden cardiac death of *trans* C18:1 and C18:2 fatty acids combined did not differ significantly from 1·0 in relation to the distribution of these *trans* isomers by quintile in the control population. The relative risk (95% CI) of sudden cardiac death in the top quintile was 0·40 (0·15–1·02) for C18:1 and 1·08 (0·48–2·74) for C18:2 compared with the bottom quintiles of their respective control distributions. When these univariate relations for *trans* fatty acids were adjusted for coronary risk factors, smoking was the only factor that remained independently associated with risk of sudden cardiac death (2·27 [1·23–4·17]).

Overall, there was no evidence of a relation between *trans* isomers of oleic and linoleic acids combined and sudden cardiac death. However, *trans* oleic acid was negatively associated with risk of sudden cardiac death, whereas no association with *trans* forms of linoleic acid was seen. This study does not support the hypothesis that *trans* isomers increase the risk of sudden cardiac death.

Lancet 1995; **345:** 278–82

See Commentary page 269

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Introduction

Carbon-to-carbon double bonds of unsaturated fatty acids have two potential geometric isomer configurations, *cis* and *trans,* and the prefix indicates whether the alkane chains are on the same side or opposite sides of the molecule. Geometric *trans* isomers are formed by biological or industrial hydrogenation of *cis* fatty acids in vegetable and fish oils and fats from ruminants (eg, beef fats and milk).[1] Variation in the geometric form of these fatty acids confers different biological properties, and Mann[2] has proposed that exposure to *trans* fatty acids in partly hydrogenated fats impairs lipoprotein receptors during energy surfeit, leading to hypercholesterolaemia, atherogenesis, obesity, and insulin resistance. This mechanism might explain the observed age, sex, and national differences in rates of coronary heart disease (CHD).[2]

The physical chemistry of *cis* and *trans* isomers means they can be measured by several methods, including infrared absorption spectroscopy and standard gas-liquid chromatography.[1] The first method does not lend itself readily to small biological samples and does not distinguish between different *trans* isomers. Gas-liquid chromatography with packed columns can distinguish some *trans* isomers but is not ideal. Many of the *trans* isomers of oleic (C18:1) and linoleic acid (C18:2) can, however, be resolved by high-resolution capillary gas-chromatography.[3] Dietary *trans* fatty acids come from several sources and the proportion consumed that possess double bonds in the geometric form is reflected in the composition of adipose-tissue triglyceride fatty acids.[4]

Few epidemiological studies have examined the relation between *trans* fatty acids, measured in diet or adipose tissue, and the risk of CHD, and results are contradictory. In geographic and case-control studies in the UK no significant differences in adipose *trans* fatty acids (C16:1 and C18:1) were found between subjects who had died from CHD and those who had died from other causes. Although a prospective cohort study of women from the USA[5] found that consumption of *trans* fatty acids in all forms was associated with increased risk of acute myocardial infarction or death from CHD, the relation of individual *trans* isomers to CHD—which might not all have the same biological effect—remains open. We reported an inverse relation between linoleic acid and risk of sudden cardiac death from a population case-control study of adipose-tissue triglyceride fatty-acid composition in men under the age of 65 years.[6] We have now reanalysed the adipose-tissue samples from this study to